AD-A261 492

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	ANNUAL 01 Oct 91 TO 30 Sep 92

4. TITLE AND SUBTITLE
STRESS-INDUCED ENHANCEMENT OF THE STARTLE REFLEX

5. FUNDING NUMBERS
AFOSR-91-0035
61102F
2312
A2

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8. PERFORMING ORGANIZATION REPORT NUMBER

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10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release;
distribution

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 words)

The role of the amygdala in the acquisition of conditioned fear. Conditioned fear-potentiated startle involves both learning (e.g., learning the association between the light and the shock), memory (e.g., retrieval of the association that the light predicts shock which then leads to a state of fear), and performance (e.g., the state of fear elevating the startle reflex). Work prior to that supported by the Air Force had purposely focused on performance, because we felt this was probably the simplest aspect of this paradigm and hence the one most amenable to experimental analysis. Hence, we chose drugs (e.g., diazepam, buspirone) or lesions (e.g., of the central nucleus of the amygdala) which should reduce fear and thereby prevent fear-enhancement of startle. This work showed that the central nucleus of the amygdala, and its direct projection to a particular part of the acoustic startle pathway, were critically involved in the performance or expression of fear-potentiated startle.

93-04645

14. SUBJECT TERMS

15. NUMBER OF PAGES

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
(U)	(U)	(U)	(UL)

annual
Final Technical Report
Number: AFOSR-91-0035
Title: Stress-Induced Enhancement of the Startle Reflex
PI: Michael Davis

A major goal of the work funded by the Air Force has been to evaluate the role of the amygdala in both conditioned and unconditioned fear and anxiety.

A. The role of the amygdala in the acquisition of conditioned fear

Conditioned fear-potentiated startle involves both learning (e.g., learning the association between the light and the shock), memory (e.g., retrieval of the association that the light predicts shock which then leads to a state of fear), and performance (e.g., the state of fear elevating the startle reflex). Work prior to that supported by the Air Force had purposely focused on performance, because we felt this was probably the simplest aspect of this paradigm and hence the one most amenable to experimental analysis. Hence, we chose drugs (e.g., diazepam, buspirone) or lesions (e.g., of the central nucleus of the amygdala) which should reduce fear and thereby prevent fear-enhancement of startle. This work showed that the central nucleus of the amygdala, and its direct projection to a particular part of the acoustic startle pathway, were critically involved in the performance or expression of fear-potentiated startle.

Work supported by the Air Force over the last several years attempted to evaluate the role of the amygdala in the actual learning process. This was done by testing whether drugs known to block the development of long-term potentiation, a candidate cellular mechanism for learning (i.e., N-methyl-D-aspartate antagonists) would block the acquisition of fear-potentiated startle.

1. The role of the lateral and basolateral amygdala nuclei in fear-potentiated startle. Our original work focussed on the central nucleus of the amygdala because of its direct projections to the acoustic startle pathway and the fact that many other studies in the literature had found it to be important for the expression of conditioned fear. However, most sensory information (e.g., carrying visual or auditory CS information) enters the amygdala through its lateral and basolateral nuclei (Amaral, 1987; LeDoux et al., 1990a; Ottersen, 1980; Turner, 1981; Van Hoesen, 1981). In turn, these nuclei project to the central nucleus (Amaral, 1987; Aggleton, 1985; Krettek & Price, 1978b; Millhouse & DeOlmos, 1983; Niteka & Frotscher, 1989; Nitecka et al., 1981; Ottersen, 1982; Roberts et al., 1982; Russchen, 1982; Smith & Millhouse, 1985), which, as discussed above, then projects directly to the acoustic startle pathway. Recent evidence indicates that the lateral nucleus of the amygdala provides a critical link for relaying auditory information to the central nucleus of the amygdala involved in fear conditioning using an auditory conditioned stimulus (LeDoux et al.,1990). Hence, we wondered how lesions of the lateral and basolateral

nuclei would affect both the acquisition and expression of fear-potentiated startle (Sananes & Davis,1992). Selective destruction of the lateral and basolateral nuclei was accomplished by local infusion of neurotoxic doses of N-methyl-D-aspartate (NMDA) into the basolateral nucleus (Hatfield et al., 1992)

NMDA lesions of the lateral and basolateral nuclei caused a complete blockade of fear-potentiated startle in each of the nine animals when the lesions were made before training. Histological examination of the nine animals indicated that six rats were judged to have complete, bilateral lesions of the lateral and basolateral nucleus. In addition, in most animals there was also damage to the dorsal endopiriform nucleus. Partial damage of the amygdalostriatal transition zone, medial aspects of the perirhinal cortex, and the ventral endopiriform nucleus was seen in some animals, although this was typically only unilateral. Most animals had sparing of the basomedial nucleus and ventral basolateral nucleus and all animals had sparing of the central nucleus of the amygdala. In the remaining three animals, a similar pattern of damage was seen except for obvious sparing of the most anterior regions of at least one basolateral nucleus.

In these studies the NMDA lesions were performed before training, so that the blockade of potentiated startle could have resulted from a blockade of acquisition or a blockade of the expression of potentiated startle, or both. In a subsequent study, NMDA lesions of the lateral and basolateral nuclei were also found to block the expression of fear-potentiated startle, because they caused a complete blockade of fear-potentiated startle when the lesions were made after training. This blockade of performance or retrieval did not seem to be attributable to a disruption of vision, because a control study showed these lesions did not disrupt visual prepulse inhibition, which requires intact visual processing. Moreover, other studies found that NMDA induced lesions of these amygdaloid nuclei also blocked the expression of fear-potentiated startle using an auditory CS.

2. <u>The role of NMDA receptors in the amygdala during the acquisition of conditioned fear</u>.

During this period, a great deal of data were coming out to show that NMDA antagonists blocked LTP *in vitro* and perhaps learning *in vivo*, based on data from several behavioral tasks. However, no studies had tested whether local infusion of NMDA antagonists into the amygdala would block the acquisition of conditioned fear. LTP can occur in amygdala brain slices (Chapman et al., 1990) or in vivo following tetanic stimulation of the part of the medial geniculate nucleus that projects to the lateral nucleus of the amygdala (Clugnet & LeDoux, 1990). If convergence between the CS and shock occurs at the amygdala, and an NMDA-dependent process is involved in the acquisition of conditioned fear, then local infusion of NMDA antagonists into the amygdala should block the acquisition of conditioned fear measured with the fear-potentiated startle effect.

To test this, rats were implanted with bilateral cannulae in the basolateral amygdaloid nucleus, which contains relatively high concentrations of NMDA receptors (Monaghan & Cotman, 1985). It was found that local infusion of the NMDA antagonist AP5 into the basolateral nucleus immediately before training caused a dose-dependent blockade of the acquisition of fear-potentiated using either a visual (Miserendino, Sananes, Melia & Davis, 1990) or an auditory (Campeau, Miserendino, & Davis, 1992) conditioned stimulus. Observation of the animals during training found no evidence of catalepsy or ataxia (e.g., Leung & Descorough, 1988). The effect did not seem to result from a decrease in sensitivity to footshock, because local infusion of AP5 into the amygdala did not alter either overall reactivity to footshock or the slope of reactivity as a function of different footshock intensities. On the other hand, AP5 did not block the expression of fear-potentiated startle when it was infused immediately prior to testing in rats previously trained in the absence of the drug. Interestingly, however, pre-testing infusion of the non-NMDA receptor antagonist CNQX did block the expression of fear-potentiated startle in a dose-dependent manner (Kim, Campeau, Falls & Davis, in press). This suggests that the CS ultimately releases glutamate in the amygdala which activates non-NMDA receptors for the expression of conditioned fear. Moreover, this finding makes it more difficult to ascribe the fear acquisition deficit observed with AP5 to non-specific antagonism of the non-NMDA receptors, because AP5, unlike CNQX, did not block the expression of fear-potentiated startle.

Other studies showed that AP5 given after training, but 1 week before testing, did not block potentiated startle, ruling out any permanent damage to the amygdala or blockade caused by residual drug during testing. Finally, infusion of AP5 into deep cerebellar nuclei did not block acquisition even at a dose 8 times that required to block acquisition after local infusion into the amygdala.

The ability of AP5 locally infused into the amygdala to block the acquisition but not the expression of conditioned fear has now been replicated using other measures of fear such as freezing (Fanselow, Kim, & Landeira-Fernandez, 1991) or inhibitory avoidance (Kim & McGaugh, 1992; Liang & Davis, submitted). Together, these results suggest the involvement of NMDA receptors in the lateral/basolateral amygdaloid nuclei in the acquisition of aversive conditioning. It is unlikely that the observed acquisition deficit is mediated by a blockade of NMDA receptors located on central amygdaloid neurons because acquisition of conditioned freezing is not blocked by direct infusion of AP5 in this nucleus (Fanselow, Kim, & Landeira-Fernandez, 1991).

B. The role of the amygdala in the inhibition of fear

Although a great deal of progress has thus been made in determining the neural systems involved in the acquisition and expression of fear and anxiety, practically nothing is known about the neural systems that may be involved in the reduction or elimination of conditioned fear.

Clinically, the inability to eliminate fear and anxiety ranks as one of the major problems in psychiatry. Hence, it would be important to develop methods to begin to identify brain systems involved in the inhibition of fear.

1. The role of NMDA receptors in the amygdala during the acquisition of extinction. There are many examples in the behavioral literature of learning-induced changes that involve the reduction or elimination of conditioned fear. Experimental extinction is a primary example. To begin to identify neural systems that might be involved in the reduction of conditioned fear, we asked whether blockade of NMDA receptors at the level of the amygdala would alter the process of experimental extinction (Falls, Miserendino & Davis, 1992). Rats were implanted with bilateral cannulae aimed at the basolateral nucleus of the amygdala and trained for potentiated startle in the usual way. One week later, all animals were given an initial short test session and subsequently matched into four groups each having equivalent levels of fear-potentiated startle. The next day half the animals were presented with 30 light-alone trials in which shocks were omitted. Five minutes before this extinction session, one group was infused with AP5 and one group with artificial CSF vehicle. The two remaining groups were treated identically, except no lights were presented. They were placed into the test cages immediately after receiving either AP5 or artificial CSF. Twenty-four hours later, all rats were tested for fear-potentiated startle.

Animals infused with AP5 or artificial CSF, but not given light-alone trials, had levels of potentiated startle equivalent to that observed in their initial test. Animals infused with artificial CSF immediately before light-alone trials had very little potentiated startle on their second test, indicating that extinction had occurred. In contrast, animals infused with AP5 immediately before light-alone trials had levels of potentiated startle that did not differ from their initial test or from the groups infused with either artificial CSF or AP5, but not given light-alone trials, and significantly higher levels than the group given artificial CSF and light-alone trials. These data indicate, therefore, that AP5 infused into the amygdala blocked extinction of fear-potentiated startle suggesting that an NMDA dependent mechanisms in, or close to, the amygdala may be important for extinction of conditioned fear.

2. Conditioned inhibition. Extinction has been considered a special case of the more general phenomenon of conditioned inhibition (c.f., Bouton, 1991). In the typical conditioned inhibition procedure, one stimulus, denoted as A, predicts shock while another stimulus X, predicts the absence of shock. The result of this procedure is that A comes to elicit a fear reaction when presented alone but not when it is accompanied by X, the conditioned inhibitor. Extinction may be analogous to conditioned inhibition in that the experimental context is like X in that it predicts the absence of shock (c.f. Bouton and Bolles, 1985, Bouton and King, 1983, Bouton and King, 1986). The conditioned inhibition procedure, however, offers advantages over the extinction procedure. In the conditioned inhibition procedure, the reduction of fear is under the

control of an explicit CS, rather than under the control of contextual cues. Moreover, fear reduction is assessed at the same time as fear production, allowing one to disentangle the inhibition of fear from a more global disruption in fear performance or stimulus processing.

Because of the possible advantages of the conditioned inhibition procedure, we have devised a procedure for obtaining conditioned inhibition of fear-potentiated startle. Rats underwent two days of training in which one stimulus, denoted as A+, was repeatedly paired with footshock. Following this, the rats underwent five additional days of training in which a serial compound, denoted as X*A-, was not paired with shock. A+ training was continued during this second phase. Conditioned inhibition was assessed by measuring the amplitude of the startle reflex in the presence or absence of A when it was or was not preceded by X (i.e., A or X*A).

Figure XX shows substantial fear to A as evidenced by greater startle amplitude in the presence of A than in its absence. However, the rats showed significantly less fear-potentiated startle to A when it was preceded by X. This suggests that X had acquired the ability to inhibit the fear produced by A. This inhibitory effect of X was dependent on the rats having been given explicit non-reinforced presentations of the serial X*A compound, because, in a control experiment, rats given X alone trials in the second phase of training did not show inhibition of fear-potentiated startle to A when preceded by X in testing. Moreover, the lack of potentiated startle on X*A trials cannot be readily attributed to a configural discrimination (i.e., A vs. XA) because in a subsequent experiment, the inhibitory effect of X transferred to another fear-eliciting stimulus, C, such that fear-potentiated startle to X*C was less than that to C alone. Taken together, these results suggest that X in a A+/X*A- procedure acquires the ability to inhibit fear-potentiated startle.

Because so much is known about the neural systems mediating the acquisition and performance of fear-potentiated startle, we are now in a position to begin to ask what structures may be responsible for the reduction of conditioned fear. In light of the finding that extinction of conditioned fear-potentiated startle seems to involve an NMDA dependent mechanism in, or close to, the amygdala, we are currently investigating whether a similar mechanism underlies conditioned inhibition of fear-potentiated startle. In addition, we have begun to ask whether structures extrinsic to the amygdala are critical for either extinction or conditioned inhibition of fear-potentiated startle.

Publications:

A. Original articles

Melia, K.M. & Davis, M. Effects of septal lesions on fear-potentiated startle and the anxiolytic effects of buspirone and diazepam. Physiology and Behavior. 1991, 49, 603-612.

Rosen, J.B., Hitchcock, J.M., Sananes, C.B., Miserendino, M.J.D. and Davis, M. A direct projection from the central nucleus of the amygdala to the acoustic startle pathway: Anterograde and retrograde tracing studies. Behavioral Neuroscience, 1991, 105, 817-825.

Hitchcock, J.M. and Davis, M. Efferent pathway of the amygdala involved in fear conditioning using the fear-potentiated startle paradigm. Behavioral Neuroscience, 1991, 105, 826-842.

Campeau, S., Hayward, M.D., Hope,B.T., Rosen, J.B., Nestler, E.J. and Davis, M. Induction of the *c-fos* proto-oncogene in rat amygdala during unconditioned and conditioned fear. Brain Research, 1991, 565, 349-352.

Grillon, C, Ameli, R., Woods, S., Merikangas, K. and Davis, M. Fear-potentiated startle in humans: Effect of anticipatory anxiety on the acoustic blink reflex. Psychophysiology, 1991, 28, 588-595.

Kehne, J.H., Boulis, N.M. and Davis, M. Effects of the phosphodiesterase inhibitor rolipram on the acoustic startle reflex. Psychopharmacology,1991, 105, 27-36.

Sananes, C.B. and Davis, M. NMDA lesions of the lateral and basolateral nuclei of the amygdala block fear-potentiated startle and shock sensitization of startle. Behavioral Neuroscience, 1992, 106, 72-80.

Melia, K. R.,Sananes, C.B. and Davis, M. Lesions of the central nucleus of the amygdala block the excitatory effects of septal ablation on the acoustic startle reflex. Physiology & Behavior, 1991, 51, 175-180.

Falls, W.A., Miserendino, M.J.D., and Davis, M. Extinction of fear-potentiated startle: Blockade by infusion of an NMDA antagonist into the amygdala. Journal of Neuroscience, 1992, 12, 854-863.

Campeau, S. and Davis, M. Fear potentiation of the acoustic startle reflex using noises of various spectral frequencies as conditioned stimuli. Animal Behavioral and Learning, 1992, 20, 177-186.

Campeau, S., Miserendino, M.J.D. and Davis, M. Intra-amygdala infusion of the N-methyl-D-aspartate receptor antagonist AP5 blocks acquisition but not expression of fear-potentiated startle to an auditory conditioned stimulus. Behavioral Neuroscience, 1992, 106, 569-574.

Liang, K.C., Melia, K.R., Miserendino, M.J.D., Falls, W. A ., Campeau, S. and Davis, M. Corticotropin-Releasing Factor: Long-lasting facilitation of the acoustic startle reflex. Journal of

Neuroscience, 1992, 12, 2303-2312.

Liang, K.C., Melia, K.R., Campeau, S., Falls, W. A ., Miserendino, M.J.D., and Davis, M. Lesions of the central nucleus of the amygdala, but not the paraventricular nucleus of the hypothalamus block the excitatory effects of corticotropin-releasing factor on the acoustic startle reflex. Journal of Neuroscience,1992, 12, 2313-2320

Melia, K.R., Falls, W.A., & Davis, M. Involvement of pertussis toxin sensitivie G-proteins in conditioned fear-potentiated startle: Possible involvement of the amygdala. Brain Research,1992, 584, 141-148.

B. Reviews and Chapters

Davis, M. The role of the amygdala in fear and anxiety. In: The Annual Review of Neuroscience, 15, 1992, 353-375.

Davis, M. The role of the amygdala in conditioned fear. In: The Amygdala: Neurobiological Aspects of Emotion, Memory, and Mental Dysfunction (John Aggleton, Ed.) 1992, Wiley-Liss, Inc. New York, 255-305.

Davis, M. Animal models of anxiety based on classical conditioning: The conditioned emotional response (CER) and the fear-potentiated startle effect. In: The International Encyclopedia of Pharmacology and Therapeutics, S.E. File (Ed)., 1991, Pergamon Press, New York. 187-212.

Davis, M., Hitchcock, J.M., and Rosen, J.B. Neural mechanisms of fear conditioning measured with the acoustic startle reflex. In" Neurobiology of Learning, Emotion, and Affect, J. Madden IV, (Ed), 1991, Raven Press, Ltd. New York, 67-95.

Davis, M. The role of the amygdala in fear-potentiated startle: Implications for animal models of anxiety. Trends in Pharmacological Sciences, 1992, 13, 35-41.

Davis, M., Hitchcock, J.M., and Rosen, J.B. A neural analysis of fear conditioning. In: Learning and memory: The behavioral and biological substrates. I. Gormezano and E.A. Wasserman (Eds). Erlbaum Press, Hillsdale, N.J., 1992, pp 153-182

Davis, M. Analysis of aversive memories using the fear-potentiated startle paradigm. In: Neuropsychology of Memory N. Butters & L. Squire (Eds), Guilford Press: New York, 1992, pp. 470-484.

Davis, M. A Neural Systems Approach to the Study of the Amygdala, Fear and Anxiety

In: Experimental Approaches to Anxiety and Depression, M. Elliott (Ed)., John Wiley & Son, Inc. New York, 1992, pp. 43-71.